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LETTERS

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Detection of *Neisseria gonorrhoeae* by PCR using *orf*1 gene as target

Nucleic acid amplification tests have the ability to specifically amplify small quantities of DNA and hence have been used successfully in the diagnosis of STDs.[1 2] An in-house polymerase chain reaction (PCR) method was developed and evaluated for the detection of *Neisseria gonorrhoeae* DNA in the urogenital specimens collected (with consent) from patients visiting an STD clinic in India.

The primers (forward primer 5′-CAACTATTCCCGATTGCGA-3′ and reverse primer 5′-GTTATACAGCTTCGCCTGAA-3′) amplify the 221–480 bp region of *orf*1 gene. Clinical isolates (n = 40) of *N gonorrhoeae* were recovered from urethral or cervical swabs by inoculation onto modified Thayer-Martin medium and identified by Gram stain, colony morphology, positive oxidase, and rapid carbohydrate utilisation test. For PCR the clinical samples (n = 489) were centrifuged (30 minutes, 14 000 *g*) and the cell pellet was lysed with 50 mM TRIS-HCl (pH 7.5) 1% Triton X-100, 1 mM EDTA, 250 µg of proteinase K per ml at 37° for 1 hour, boiled for 10 minutes, and centrifuged. Eight µl of lysate was used for amplification (40 cycles) under standard conditions. Each cycle consisted of 30 seconds at 94°C, 30 seconds at 52°C, and 1 minute at 72°C. The amplified PCR product (10 µl) was analysed by electrophoresis in a 2% agarose gel and characterised by sequencing.

An amplified product of 260 base pairs (bp) of *orf*1 gene was observed with all *N gonorrhoeae* isolates but not when DNA from the other non-gonococcal strains (17 closely related *Neisseria* species, *Corynebacterium*, *Chlamydia trachomatis*, *Candida*, syphilis, and members of Enterobacteriaceae) was used as template. For the 427 clinical swabs collected from men, 379 were positive and 46 were negative by both culture method and *orf*1-PCR assay. Urethral specimens from two men were culture negative but PCR positive for *orf*1 gene. Since these two samples tested PCR positive for *cpp*B gene of *N gonorrhoeae*[3] they were considered true positives. Thus, a total of 381 men (89%) were classified as true positives based on the PCR assay (table 1). Of the 62 women tested, 52 were true positives, and five were true negative as they gave concordant results irrespective of the site of collection and the diagnostic method used (table 1). Four culture negative specimens tested positive by the PCR assays using primers specific to *orf*1 as well as *cpp*B gene and were, therefore, considered positive. One culture negative specimen was positive by the *orf*1-PCR assay for its endocervical specimen but negative for urethral specimens. For the *cpp*B gene amplification, the specimen yielded a negative result for both the sites. This was therefore classified as true negative. The sensitivity, specificity, positive predictive value, negative predictive value for the PCR method described here would be 100%, 98%, 99.7%, and 100% respectively. The gold standard has been reported as having a sensitivity of 85–95%.[4 5]

The high specificity and sensitivity (25 fg DNA per assay, equivalent to 10 cells) coupled with low cost and rapidity of the in-house PCR assay described here can serve as a promising diagnostic method for the detection of gonococcus directly from clinical swab samples.

Table 1 Comparison of culture and PCR method for detection of *Neisseria gonorrhoeae* in urogenital specimens from men and women

No of specimens from men	Urethra				Patient status
	Culture	Gram stain	PCR (*orf*1/*cpp*B gene)		
46	–ve	–ve	–ve		Not infected
367	+ve	+ve	+ve		Infected
12	+ve	–ve	+ve		Infected
2	–ve	–ve	+ve/+ve		Infected
No of specimens from women	**Urethra**		**Endocervix**		
	Culture	**PCR**	**Culture**	**PCR**	**Patient status**
5	–ve	–ve	–ve	–ve	Not infected
52	+ve	+ve	+ve	+ve	Infected
1	–ve	–ve	–ve	+ve	Not infected*
4	–ve	+ve	–ve	+ve	Infected

*The individual was categorised as not infected after confirming with the *cpp*B gene PCR.

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References

1 **Farrell DJ**. Evaluation of Amplicor Neisseria gonorrhoeae PCR using cppB nested PCR and 16S rRNA PCR. *J Clin Microbiol* 1999;**37**:386–90.
2 **Kehl SC**, Georgakas K, Swain GR, *et al*. Evaluation of the Abbott LCx assay for detection of Neisseria gonorrhoeae in endocervical swab specimens from females. *J Clin Microbiol* 1998;**36**:3549–51.
3 **Ho BS**, Feng WG, Wong BK, *et al*. Polymerase chain reaction for the detection of Neisseria gonorrhoeae in clinical samples. *J Clin Pathol* 1992;**45**:439–42.
4 **Hook EW III**, Handsfield HH. Gonococcal infections in the adult. In: Holmes K, Straling F, Wiesner P, Cates W, Lemon S, Stamm W, ed. *Sexually transmitted diseases*. New York: McGraw Hill, 1990:149–65.
5 **Ching S**, Lee H, Hook E, *et al*. Ligase chain reaction for detection of Neisseria gonorrhoeae in urogenital swabs. *J Clin Microbiol* 1995;**33**:3111–14.

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Nevirapine + efavirenz based salvage therapy in heavily pretreated HIV infected patients

The emergence of protease inhibitors (PIs) and multiple drug therapy for HIV infection has greatly decreased mortality in countries where these medications are available. Unfortunately, many patients eventually develop viral resistance to treatment because of HIV virus mutations. As clinicians await development of new drugs to combat resistant virus, innovative strategies with existing drugs may be particularly valuable. Patients having failed regimens containing nucleoside reverse transcriptase inhibitors (NRTIs) and PIs face limited options for future therapy. A regimen containing the two potent non-nucleoside reverse transcriptase inhibitors (NNRTIs), nevirapine (NVP) and efavirenz (EFV), could provide an effective alternative, since both can be conveniently dosed once daily[1 2] and have demonstrated efficacy in patients with high viral loads.[2–4]

A retrospective chart review at an urban HIV hospital clinic identified 13 patients who had initiated an NVP + EFV based salvage

Table 1 Baseline characteristics and outcome for NNRTI naive and experienced patients

Patient No	Regimen	Previous drugs	Baseline viral load (copies/ml)	Baseline CD4+ cell count (cells $\times 10^6$/l)	Months of follow up	Last viral load (copies/ml)
1	NVP+EFV+d4T	d4T, ddI, RTV, IDV, ABC	37100	290	15	164000
2	NVP+EFV+d4T	NFV, CBV, IDV	436000	183	7	<50
3	NVP+EFV+ABC	IDV, d4T, NFV, ddC	27000	–	13	<50
4	NVP+EFV+d4T	CBV, NFV, SQV	151000	161	9	<50
5	NVP+EFV+d4T	NFV, CBV	31900	392	18	<50
6	NVP+EFV+d4T+ddI	d4T, ddI, NFV	5000	440	6	<50
7	NVP+EFV+RTV+IDV	IDV, NFV, d4T, CBV	750000	20	10	<50
8	NVP+EFV+d4T	NFV, CBV	750000	2	11	<50
9	NVP+EFV+ddI	IDV, ddI	–	–	12	<50
10	NVP+EFV+ddI	RTV, other PI	35900	180	3	<50
11*	NVP+EFV+RTV+IDV	DLV, RTV, d4T	3100	190	14	<50
12*	NVP+EFV+RTV+IDV	SQV, NFV, NVP, ddI, DLV	8900	276	13	<50
13*	NVP+EFV+RTV+IDV	SQV, d4T, ddI, EFV, IDV	3900	235	11	33000

*NNRTI experienced patients.
NPV = nevirapine, EFV = efavirenz, d4T = Stvudine, ABC = abacavir, ddI = didanosine, RTV = ritonavir, IDV = indinavir, CBV = carbovir, SQV = saquinavir, DLV = delaviridine.

regimen (table 1). Inclusion of these patient charts in this study was approved by a research ethics committee at Bellevue Hospital. All patients received NVP + EFV at standard doses. The lower limit of quantitation was determined at 50 HIV RNA copies/ml using Roche Amplicor HIV-1 Monitor (RNA) (Roche Diagnostics, Branchburg, NJ, USA). Median baseline values were: viral load 33 900 copies/ml (range 3100–750 000 copies/ml) and CD4+ count 190 cells $\times 10^6$/l (range 2–440 cells). After a median follow up of 11 months (range 3–18 months), 85% (11/13) had viral loads <50 copies/ml. Considering previous treatment experience, 90% (9/10) of NNRTI naive patients had viral loads <50 copies/ml and 67% (2/3) of NNRTI experienced patients had viral loads <50 copies/ml. Effectiveness of the dual NNRTI combination in heavily pretreated patients is in contrast with a study using a single NNRTI plus two NRTIs in NRTI experienced patients in whom rapid virological failure was observed.[5] These results suggest that the combination of two potent NNRTIs may be able to overcome development of NNRTI associated resistance, even when there are only one or two NRTIs in the combination. These data accord with those of Jordan and colleagues who demonstrated a sustained response to NVP + EFV in combination with only didanosine (ddI) in 19/21 patients after 12 months.[6]

The most common adverse event was elevated liver function test results (more than three times upper limit of normal) in three patients. One case of liver toxicity was attributed to Bactrim, and a second case resolved following interruption of EFV (EFV rechallenge in this patient was successful). No specific cause of liver toxicity could be identified in the third case, suggesting a possible association with antiretroviral treatment. Other adverse events included anaemia. The patient with EFV induced hepatotoxicity also had anaemia and EFV related central nervous system disturbances. None of the patients discontinued therapy because of adverse events. The relatively low incidence of adverse events and the absence of NNRTI associated metabolic disorders make this dual NNRTI based regimen additionally appealing.

This retrospective analysis demonstrated the effectiveness of the combination of two NNRTIs (NVP and EFV) in heavily pretreated PI experienced patients, with no apparent increase in NNRTI related side effects. Since few new antiretrovirals with novel resistance profiles are forthcoming in the near future, this regimen may provide a much needed alternative in heavily pretreated patients.

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References

1 **Van Heeswijk RPG**, Veldkamp AI, Mulder JW, *et al*. The steady-state pharmacokinetics of nevirapine during once daily and twice daily dosing in HIV-1-infected individuals. *AIDS* 2000;**14**:F77–82.
2 **Staszewski S**, Morales-Ramirez J, Tashima KT, *et al*. Efavirenz plus zidovudine and lamivudine, efavirenz plus indinavir, and indinavir plus zidovudine and lamivudine in the treatment of HIV-1 infection in adults. *N Engl J Med* 1999;**341**:1865–73.
3 **Pollard R**. Factors predictive of durable HIV suppression in randomized double-blind trial with nevirapine (NVP), zidovudine (ZDV), and lamivudine (3TC) in treatment-naive (ARV-n) patients with advanced AIDS. 7th Conference on Retroviruses and Opportunistic Infections, San Francisco, USA, 30 Jan–2 Feb 2000 (abstract 517).
4 **Podzamczer D**, Ferrer E, Consiglio E, *et al*. A randomized, open, multicenter trial comparing Combivir plus nelfinavir or nevirapine in HIV-infected naive patients (the Combine study). 8th Conference on Retroviruses and Opportunistic Infections, Chicago, USA, 4–8 Feb 2001 (abstract 327).
5 **D'Aquila RT**, Hughes MD, Johnson VA, *et al*. Nevirapine, zidovudine, and didanosine compared with zidovudine and didanosine in patients with HIV-1 infection. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 1996;**124**:1019–30.
6 **Jordan WC**, Jefferson R, Yemofio F, *et al*. Nevirapine (NVP) + efavirenz (EFV) + didanosine (ddI): a very simple, safe, and effective once-daily regimen. 13th International AIDS Conference, Durban, South Africa, 9–14 July 2000; abstract TuPeB3207.

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Comparing cost effectiveness of screening women for *Chlamydia trachomatis* in systematic and opportunistic approaches

Screening women for asymptomatic *Chlamydia trachomatis* (CT) infections is indicated to prevent the spread of CT and the development of complications such as pelvic inflammatory disease (PID), chronic pelvic pain, ectopic pregnancy, tubal infertility, and neonatal pneumonia (major outcomes averted; MOA). Cost effectiveness presents an important aspect in the decision making regarding actual implementation. Recently, in this journal Van Valkengoed *et al* published a paper on the cost effectiveness of systematic screening among women in Amsterdam (Netherlands), using pharmacoeconomic modelling.[1] Using the same model, results on the cost effectiveness of an opportunistic screening in the same city have also been published.[2] Specific model assumptions differed in both publications. The aim of this letter is to compare cost effectiveness of systematic and opportunistic screening using similar model assumptions and correcting for potential biases.

Opportunistic screening was done during May 1996 to May 1997 in a pilot study.[3] Women visiting the participating GPs were eligible for screening if they considered themselves heterosexually active, were aged 15–40 years, and did not visit their GP for sexually transmitted disease complaints (participation among women: 96% compared with 50% in the systematic screening). In this letter we report on the age group 15–30. Obviously, the effectiveness of this type of screening depends on the frequency of visiting the GP; 87% of Dutch women aged 15–30 visit the GP at least once per year.[2] As in the systematic universal screening, testing was done with ligase chain reaction (LCR) on urine. Participating GPs in the opportunistic screening had an overrepresentation compared to the general Amsterdam situation of participants from Caribbean and Surinam ethnicity with relatively high CT prevalence.[3] To enhance valid comparison with the systematic screening, asymptomatic CT prevalence rates in the opportunistic screening were recalculated standardising for the distribution of the Amsterdam population over the ethnic groups of Caribbean, Surinam, and other (source: Statistics Amsterdam).

Parameters in the pharmacoeconomic model were kept similar to the previous paper in this journal, except for the probability of PID after asymptomatic infection.[1] For this probability we applied 20% compared to 10% in the paper by Van Valkengoed *et al*.[1] We even consider 20% as a very conservative estimate for the risk of PID in our model.[4] Cost effectiveness was estimated as net costs per MOA in baseline analysis using assumptions